Technical Note

Identification of a Dipyrone Acetylation Reaction Product Found in Some Black-Tar Heroin Exhibits

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ABSTRACT: N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-N-methyl-acetamide was identified as an impurity in a small number of Mexican black tar heroin exhibits. The presence of this compound suggests that dipyrone was added to the morphine base prior to its acetylation with acetic anhydride. Spectroscopic and chromatographic data are provided.

KEYWORDS: Heroin, Dipyrone, Acetylated Products, Impurity Profiling, Mass Spectrometry, Forensic Chemistry.

Introduction

Analysis of four black tar heroin exhibits submitted to this laboratory were determined to contain 38.5 - 48.1% heroin, typical heroin-related alkaloids (acetyl codeine, O6-monoacetylmorphine, etc.), and an unknown compound. The unknown eluted before heroin and contained fragment ions similar to those found for a known dipyrone injection port artifact, but had a mass of 42 Daltons higher, suggesting that it was the acetylated by-product of the dipyrone artifact, or a related impurity. Levamisole and lidocaine acetylation by-products in heroin have been recently reported from direct acetylation of morphine containing these compounds [1]. In order to determine whether a similar reaction was occurring, dipyrone was subjected to an acetylation reaction (Figure 1) and the isolated by-product was analyzed by GC/MS and NMR.

Experimental

Solvents, Chemicals, and Materials
All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). All other chemicals were of reagent-grade quality and were products of Sigma-Aldrich Chemical (Milwaukee, WI). Dipyrone was acquired from from the reference collection of this laboratory.

Gas Chromatography/Mass Spectrometry
GC/MS analyses were performed using an Agilent (Santa Clara, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph. The GC system was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm DB-1 (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100°C; initial hold, 0.0 min; program rate, 6°C/min; final temperature, 300°C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and at a temperature of 280°C. The MSD was operated in the electron ionization mode at 70 eV, a scan range of 34-700 mass units, and a scan rate of 1.34 scans/s. The auxiliary transfer line to the MSD and the source were maintained at 280°C and 230°C, respectively.

Nuclear Magnetic Resonance Spectroscopy

Proton (^{1}H), carbon (^{13}C), and 2-Dimensional NMR spectra were obtained on an Agilent VNMRS 600 MHz NMR using a 5 mm broad band detection, variable temperature, pulse field gradient probe (Agilent, Santa Clara, CA). Samples were dissolved in deuterochloroform ($CDCl_3$) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound (Cambridge Isotope Laboratories, Tewksbury, MA). The sample temperature was maintained at 25°C. Standard Agilent pulse sequences were used to acquire ^{1}H, proton-decoupled ^{13}C, and gradient versions of HSQC and HMBC spectra. Data processing and structure elucidation were performed using software from Agilent and Applied Chemistry Development (ACD/Labs, Toronto, Canada).

Synthesis

N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1 H-pyrazol-4-yl)-N-methylacetamide: Dipyrone sodium salt (367 mg, 1.1 mmol) was heated at 110°C with acetic anhydride (3.0 mL, 41 mmol) in a 15 mL capped centrifuge tube for 2 hours. The reaction was cooled and quenched with 50 mL of water, washed with Et_2O (2 x 60 mL, discarded), extracted with $CHCl_3$ (2 x 8 mL), and the latter extracts were combined, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give 242 mg of a light brown powder (85% yield). The material was sufficiently pure for chromatographic and spectroscopic analyses, and was not further purified.

GC/MS Analytical Artifact Experiment

Approximately 25 mg of an exhibit containing the suspect compound was dissolved into 1 mL of water and extracted with $CHCl_3$. The extract was washed with 4 mL of 0.36N H_2SO_4, dried over Na_2SO_4, and analyzed via GC/MS.

Results and Discussion

GC/MS analysis of four heroin exhibits revealed a previously unknown peak in their total ion chromatograms (Figure 2a, Table 1). This compound (Peak #1) had an apparent molecular ion at *m/z* 259 (Figure 3a), and appeared to be related to a dipyrone injection port artifact (i.e., from cleavage of the methanesulfonic acid moiety) based on the presence of ions found at *m/z* 56, 83, 123, and 217 (Figure 3b). Further examination showed an ion at *m/z* 43 that is indicative of an acetyl loss. The mass spectral data suggested that the compound was an acetylated dipyrone product. Dipyrone was acetylated as outlined in the experimental section and produced a single compound, with an identical mass spectrum to peak #1. Analysis via NMR (Table 2) and GC/MS identified the compound as N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-N-methyl-acetamide.

In order to demonstrate that the title compound was not formed as an injection port artifact via trans-acetylation with heroin, a heroin exhibit was extracted (see Experimental) to remove all heroin, and the remaining material re-analyzed via GC/MS. The resulting chromatographic profile confirmed that the acetylated dipyrone product was still present, thereby eliminating the possibility of trans-acetylation (Figure 2b).

Conclusion

Characterization of the dipyrone acetylation product present in the heroin exhibits, in concert with the performed acetylation experiments, verify that dipyrone was added to the morphine prior to its acetylation.

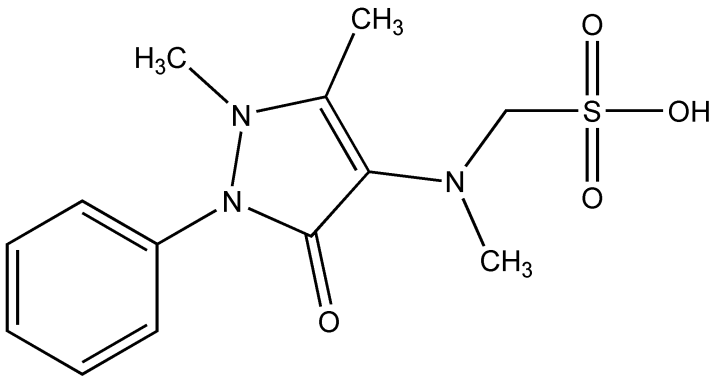
Acknowledgment

The authors are indebted to Patrick A. Hays of this laboratory for his assistance in acquiring the NMR data.

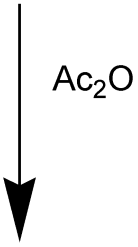
References

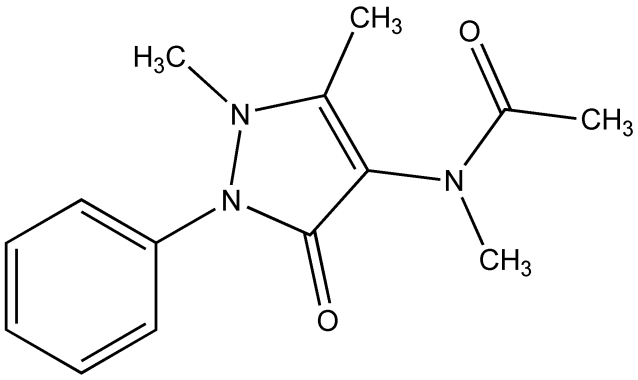
1. Casale EM, Casale JF. Identification of levamisole and lidocaine acetylation reaction impurities found in heroin exhibits. Microgram Journal 2011;8(1):16-23.

Figure 1. Structural Formulae of Dipyrone and its Acetylation Product.



Dipyrone





N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl-)N-methylacetamide

Figure 2. Partial reconstructed total ion chromatograms of heroin exhibits. Upper (a) heroin exhibit containing dypyrone acetylation by-product, and lower (b) heroin exhibit containing dipyrone acetylation by-product after removing heroin via extraction. For peak identification, see Table 1.

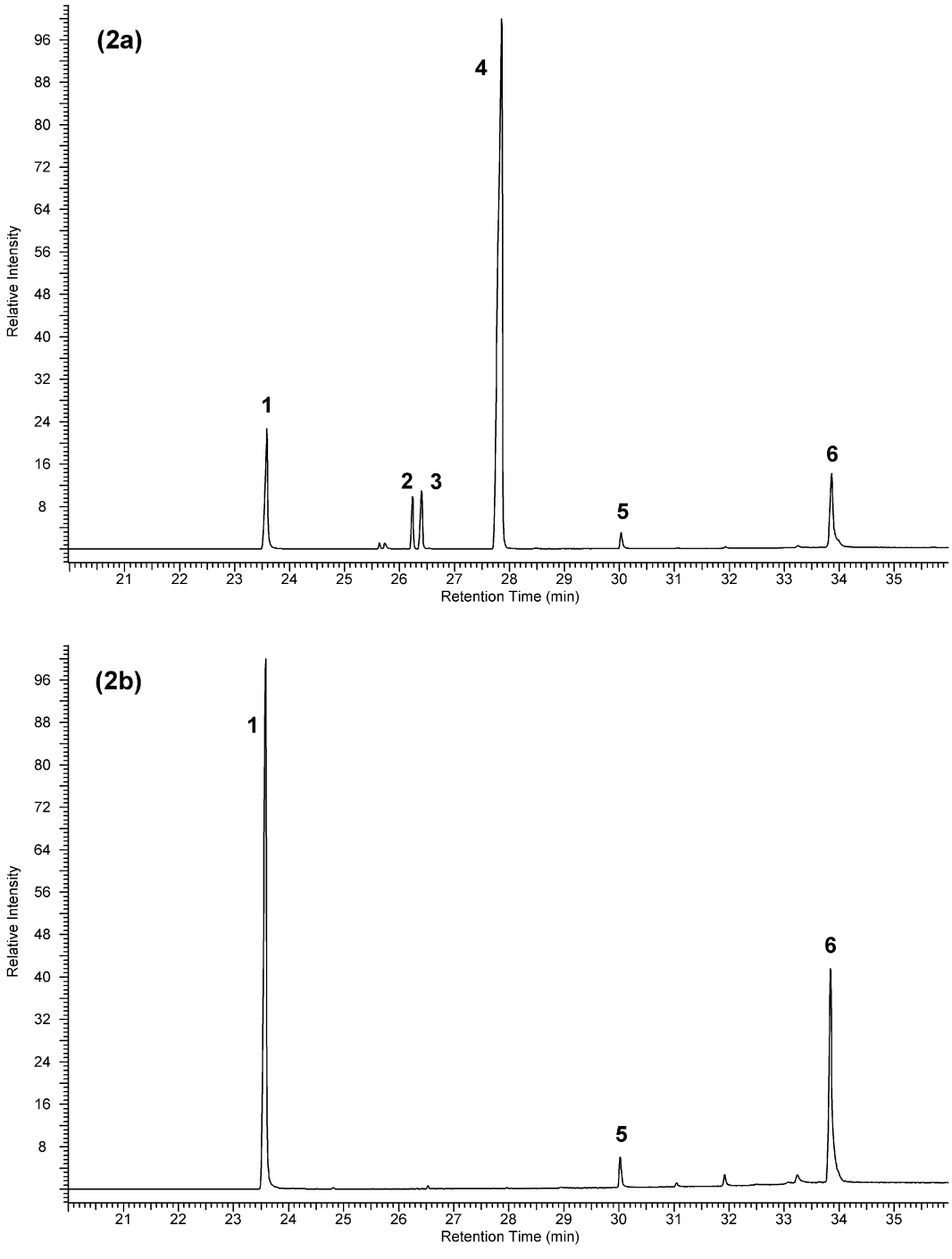


Table 1. Retention Times (RT) and Relative Retention Times (RRT) of the dipyrone acetylation product and heroin-related compounds [a].

Compound	RT (min)	RRT (min)	GC/MS Peak #
259 compound [b]	23.58	0.85	1
acetylcodeine	26.24	0.94	2
O6-monoacetylmorphine	26.40	0.95	3
heroin	27.85	1.00	4
papaverine	30.03	1.08	5
noscapine	33.86	1.21	6

[a] Conditions given in the Experimental section.
[b] N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-N-methylacetamide.

Table 2. NMR assignments of the dipyrone acetylation product dissolved in $CDCl_3$ at 600 MHz ^{1}H, 150 MHz ^{13}C.

	Carbon (ppm)	Proton (ppm)	
phenyl 1	134.4	-	1 C
phenyl *ortho*	124.3	7.40 d	2 CH
phenyl *meta*	129.3	7.48 t	2CH
phenyl *para*	127.2	7.34 t	1 CH
pyrazole C3	161.6	-	1 C
pyrazole C4	115.6	-	1 C
pyrazole C5	151.4	-	1 C
pyrazole N1-CH_3	35.6	3.15 s	1 CH3
pyrazole C5-CH_3	10.5	2.23 s	1 CH3
CH_3-C(=O)-N-**$\underline{CH_3}$**	35.8	3.15 s	1 CH3
CH_3-**$\underline{C}$**(=O)-N-CH_3	172.2	-	1 C
$\underline{CH_3}$-C(=O)-N-CH_3	21.5	2.00 s	1 CH3

Proton Multiplicity Notes: d = doublet, s = singlet, t = triplet

Figure 3. Electron ionization mass spectrum of (a) N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-N-methylacetamide; and (b) dipyrone injection port artifact.